

REVIEW ARTICLE

The Role of STAT5 in Tyrosine Kinase Inhibitor (IMATINIB) Resistance in CML Patients

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ABSTRAK

Leukemia myeloid kronis (CML) adalah kelainan sel punca hemopoietik klonal dengan translokasi resiprokal dalam kromosom 9 (ch9) dan 22 (ch22) yang menyebabkan fusi cluster Break-Abelson murine leukemia (BCR-ABL) onkogen. Penggabungan ini akan mengaktifkan tirosin kinase. Imatinib mesylate adalah inhibitor tirosin kinase (TKI) pertama yang dapat mengubah prognosis pasien CML. Namun, ada penolakan terhadap TKI, dan berdasarkan studi transkriptomik, peningkatan ekspresi transduser sinyal gen dan aktivator transkripsi (STAT) 5A dan faktor transkripsi terkait runt (RUNX3) dapat menyebabkan resistansi terhadap TKI. Protein STAT5, yang dalam sel-sel myeloid normal diaktifkan oleh sitokin, pada pasien CML diaktifkan bahkan tanpa sitokin. STAT5 merujuk pada STAT5A dan STAT5B, namun mereka mungkin memiliki peran yang berbeda dalam sel induk hematopoietik atau dalam sel CML. Ulasan ini merangkum peran STAT5 dalam resistensi inhibitor tirosin kinase pada pasien CML.

Kata kunci: leukemia myeloid kronis, STAT5, Imatinib, inhibitor tirosin kinase, resisten.

ABSTRACT

Chronic myeloid leukemia (CML) is a clonal haemopoietic stem cell disorders with reciprocal translocation in chromosome 9 (ch9) and 22 (ch22) which cause the fusion of Break cluster region-Abelson murine leukemia (BCR-ABL) oncogene. This fusion will activate tyrosine kinase. Imatinib mesylate is the first tyrosine kinase inhibitor (TKI), which could change the prognosis of CML patients. However, there is a resistance to TKI's, and based on transcriptomic study, increase expression of gen signal transducer and activator of transcription (STAT) 5A and runt-related transcription factor 3 (RUNX3) can cause resistance to TKI's. The STAT5 protein, which in normal myeloid cells being activated by cytokine, in CML patients was activated even without cytokines. STAT5 refer to STAT5A and STAT5B, however they have might have different role in hematopoietic stem cells or in CML cells. This review summarizes the role of STAT5 in tyrosine kinase inhibitor resistance in CML patients.

Keywords: chronic myeloid leukemia, STAT5, Imatinib, tyrosine kinase inhibitor, resistance.

INTRODUCTION

Chronic myeloid leukemia (CML) is one of myeloproliferative neoplasm. Fusion of the Abelson murine leukemia (ABL1) gene

on chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22 in CML express as BCR-ABL1 oncogene.¹ The BCR-ABL1 through downstream signaling pathways

such as RAS, RAF, JUN kinase, MYC, and STAT activate the tyrosine kinase continually, which creating cytokine independent cell cycle.² This Highly active tyrosine kinase are aim for survival of the cells, increase cells proliferation, increase resistance against apoptosis and changes in adhesion feature.³ Since then there was so many study were done to blockade the activity of tyrosine kinase. Until in 1996 the first tyrosine kinase imatinib been found, which is imatinib mesylate (IM) (Gleevec, Novartis Pharmaceutical Corporation, NJ, USA).⁴

Since the discover of imatinib mesylate (IM) the survival of CML patients are improving, which make IM one of the first line therapy for CML.⁴ However objective measurement was needed to evaluate the response of IM treatment, such as hematologic response, cytogenetic response and molecular response. Response of the treatment need to be evaluated every 3, 6, 12 months.⁵ However there is some population of CML patients have IM resistance. Poor response of IM led to the discover of newer generation of tyrosine kinase inhibitors (TKIs) of BCR-ABL, such as dasatinib, nilotinib, and bosutinib or even new class of treatment based on the cause of the resistance.⁶ The resistance to imatinib can be differ to primary and secondary.⁴

There are several risk factor for imatinib secondary resistance such as point mutation in BCR-ABL or over expression of BCR-ABL. However, from transcriptomic study there are increase in STAT5A and RUNX3 expression.⁷ STAT5 protein is transcriptional factor in cytoplasm or cell nuclear, which can be activated even without cytokine in CML.⁴ There are four major roles of STAT5, first to stimulate cells proliferation, to increase viability of the cell, to neutralized cell death due to TKI and increase the possibility of mutation in BCR-ABL.⁴ This led to hypothesis that STAT5 have a role

in IM resistance. Recently a study discovers an inhibitor for STAT5, known as Pimozide. Resistance of IM led us to do various study with different approach, such as knowing the role of STAT5 in CML with IM resistance.

TREATMENT RESPONSE IN CHRONIC MYELOID LEUKEMIA

There are three treatment response that could be monitor while administered TKI, first hematologic response to observe the normalization of peripheral blood counts, second cytogenetic response to observe the decrease in the number of Ph-positive metaphases using bone marrow cytogenetic, and third molecular responds. The responses in molecular response divided into three, first early molecular response (EMR) which is BCR-ABL1(IS) $\leq 10\%$ at 3-6 months, second major molecular response (MMR) which is BCR-ABL1(IS) $\leq 0.1\%$ or ≥ 3 -log reduction in BCR-ABL1 mRNA from the standarize baseline, and third complete molecular response (CMR) which variably described.⁵ The prognostic value of MMR is slightly higher than complete cytogenetic response (CCyR) in foreseeing progression free survival and overall survival, however if we did longer time of follow up CCyR become more stronger indicator than MMR. Achievement of CCyR within 12 months and no progression of the diseases into acute phase CML or blast phase CML are the goal of TKI therapy.⁵ The response milestone based on National Comprehensive Cancer Network are shown in **Table 1**.⁵

PATOPHYSIOLOGY OF RESISTANCE

As we know resistance of IM are divided into primary and secondary resistance. Primary resistance is when the patient failure to achieve the response from the guideline such as NCCN. Primary resistance divided into primary

Table 1. Milestone Response⁵

BCR-ABL1 (IS)	3 months	6 months	12 months	> 15 months
>10%	Possible TKI resistance		TKI resistant	
>1-10%		TKI Sensitive disease	Possible TKI resistance	TKI resistant
$\leq 1\%$			TKI Sensitive disease	

hematologic resistance or primary cytogenic response. Secondary resistance or also known as acquired resistance happens when previously achieved the target response than subsequently lost the response. However, the mechanism of resistance divided by BCR-ABL dependent such as gene amplification or point mutations or BCR-ABL independent.⁸

The cause of primary resistance is plasma low level of IM, low level of intracellular uptake of IM and clonal evolution. Hence the cause of secondary resistance is point mutation or over expression of BCR-ABL.⁴

BCR-ABL Dependent. Point mutation in the BCR-ABL is the most common cause of secondary resistance which also responsible for treatment failure. Around 100 points mutation already found such as T315I, Y253H and F255K. T315I is the most common compare to other point mutation and seen in 4-15% patients off patients who resistance to imatinib. The active form of BCR-ABL oncoprotein can caused by point mutations it also can causing change in the imatinib binding site, to prevent binding of imatinib and can also remove critical molecules required for bonding, thus reduce its efficacy.⁸ Other BCR-ABL depended cause is amplification of the ABL-kinase. Reactivation of BCR-ABL signal transduction is another mechanism of resistance and has been associated with both BCR-ABL point mutations and gene amplification.⁸

BCR-ABL-independent. BCR-ABL independent mechanism involve increase expression of P-glycoprotein efflux pumps can cause increased efflux of the drug. Efflux of the drug also can increase by overexpression of the P-170 glycoprotein, and can reduces intracellular drug accumulation, decreased drug uptake secondary to decreased expression of the drug uptake transporter human organic cation transporter 1 (hOCT1), and activation of the alternative signaling pathway such as Ras/Raf/MEK kinase, STAT, Erk2, or SFK phosphorylation of BCR-ABL.^{4,8} STAT5 protein also thought as one of the mechanism of BCR-ABL independent for secondary IM resistant.

TRANSDUCER SIGNAL AND ACTIVATOR OF TRANSCRIPTION 5 (STAT5)

STAT protein is a transcriptional factor, which are in the cytoplasmic and nuclear cell compartments. STAT is structurally conserved and essential for carrying out multiple cellular functions in response to extracellular cytokines and growth factors signals. STAT have 6 domains, from STAT1, STAT2, STAT3, STAT4, STAT5 (STAT5A and STAT5B) and STAT6.9 STATs have two major function which is activating transcription of diverse set of genes include genes that involved in malignancy and transducing cellular signals. Because of enforced upstream kinase signals, STATs are active in hematologic malignancy especially myeloproliferative neoplasm, leukemia and solid tumors.¹⁰ In year 1996 Van Etten reported that STAT5 directly activated by CML cell induced by BCR-ABL, which usually activated by JAK pathways.¹¹

STAT5 known to have several major roles in hematopoietic and immune cell functions such as cells proliferation, differentiation, and apoptosis.¹⁰ STAT5 can be activate in normal condition usually by cytokine such as growth factor through JAK-STAT pathways but also can dysregulated in leukemic hematopoiesis or other hematologic malignancy.¹⁰ STAT5 is activated by phosphorylation of a single tyrosine residue such as Y694 in STAT5A and Y699 in STAT5B.¹² However, STAT5 negatively regulated by dephosphorylation.¹⁰

Triggered by the BCR-ABL1 oncoprotein, STAT5 have four roles to support CML. First is the capability of stimulating cell proliferation. Second to enhances cell viability by upregulation of anti-apoptotic genes such as BCLXL and MCL1. Third the ability to counteract TKI-induced cell death and last to increase the probability of acquiring BCRABL1 mutations.¹³ The last two just being discovered recently. In CML, the expression of the Bcr-abl fusion protein trigger consecutive activation of tyrosine kinase which lead to persistent activation of STAT5.¹⁰ STAT5 also can be persistently activated by JAK2 and JAK3 mutations, Src

family kinases (SFks) and in particular, c-Src through growth factor receptor signaling, which is different from the cytokine receptor signaling in the JAK pathway.¹⁴⁻⁶

Persistent activation of STAT5 related with ROS through various mechanism, the mechanism independent of JAK2.¹⁷⁻¹⁸ STAT5 signaling also promotes ROS formation by repressing expression of antioxidant enzymes including catalase and glutaredoxin-1 (Glx1) in Bcr-Abl-positive CML.¹⁰ However Cassetti et al, reported that STAT5 especially STAT5A is protective against oxidative stress, observed by the knockdown of STAT5A increased the basal level ROS production and subsequently genomic stress in CML cell lines, while STAT5B have different contribution to stress protection.¹⁹

There are two isoforms of STAT5, STAT5A and STAT5B, which have high sequence homology and have 96% similarity in the protein level.²⁰ STAT5A and STAT5B are encoded by two different gene, however both located in the chromosome 17 in human. In human STAT5A formed by 20 exons and STAT5B formed by 19 exons.⁴ The highest prevalence of STAT5A are in mammary tissue, while STAT5B are in muscles and liver. In other tissue have same level of STAT5A and STAT5B. It also been identified that location of STAT5A and STAT5B is different in CML cells, STAT5A massively located in cytoplasm and STAT5B in nucleus.²¹

There is several difference of function between STAT5A and STAT5B, such as different responds to stress and in v-ABL expressing cells STAT5B can induce STAT5A expression, hence STAT5A cannot induced STAT5B expression.^{10,19} Study by Schönlitz et al show that there is some cytoplasmic retention of STAT5A and not STAT5B in the presence of BCR-ABL.²⁰ Also from the same study show that interaction of STAT5B-BCR-ABL required for the proliferation of human cells. When there is a reduce in BCR-ABL kinase activity, up regulation of STAT5B expression may activate STAT5 signaling.²⁰ There is study shown that STAT5B may use as agent for therapeutic in leukemia with BCR-ABL-positive. Even though inhibitor specific for STAT5B are difficult to develop because the similarity with STAT5A.²² Study done by

Zhang et al, when they did transcriptomic study and found that only STAT5A and not STAT5B as a predictor of secondary imatinib resistance.²³

STAT5 as a new target therapy. Resistance of imatinib, make it necessary to find new treatment. There is an analysis done to identify inhibitors of STAT5 seek the transcriptional activity. From this approach, the neuroleptic drug pimozide was found to inhibit STAT5-dependent reporter gene expression. Pimozide is neuroleptic drug used to treat Tourette through blockage dopamine receptor. Pimozide treatment leads to the loss of expression of endogenous STAT5 target genes that can promote malignant cellular behavior, but do not affect BCR/ABL phosphorylation, unlike imatinib who can reduce the phosphorylation of STAT5 and phosphorylation of BCR/ABL.

CONCLUSION

In CML patient treatment with imatinib, which a first-generation TKI is the first line treatment, and give a good outcome. However, the response is not the same in some patients, and they were diagnosed as imatinib resistance. Resistance of imatinib divided by primary and secondary or BCR-ABL dependent or independent. STAT5 is one thought as one cause and predictor of imatinib resistance. STAT5 also have four major roles in CML cells, which is to stimulate cells proliferation, to increase viability of the cell, to neutralized cell death due to TKI and increase the possibility of mutation in BCR-ABL1. Recently a study discovers an inhibitor for STAT5, known as Pimozide which could led to discover of new target therapy for CML. However more study are needed to study the role of stat5 in tyrosine kinase inhibitor (imatinib) resistance in CML patients.

REFERENCES

1. Apperley JF. Chronic myeloid leukaemia. *Lancet*. 2015;385(9976):1447-59
2. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. *American journal of hematology*. 2018;93(3):442-59.
3. Deininger MW, Vieira S, Mendiola R, Schultheis B, Goldman JM, Melo JV. BCR-ABL tyrosine kinase activity regulates the expression of multiple genes implicated in the pathogenesis of chronic myeloid leukemia. *Cancer research*. 2000;60(7):2049-55.

4. Casetti, Luana. New roles of STAT5 factors in chronic myeloid leukemia cell maintenance. PhD diss., Université René Descartes-Paris V, 2013.
5. National Comprehensive Cancer Network. Chronic Myeloid Leukemia (Version 1.2019). https://www.nccn.org/professionals/physician_gls/PDF/cml.pdf. Accessed Nov 30, 2018.
6. Wei G, Rafiyath S, Liu D. First-line treatment for chronic myeloid leukemia: dasatinib, nilotinib, or imatinib. *J Hematol Oncol*. 2010;3(1):47.
7. Zhang WW, Cortes JE, Yao H, et al. Predictors of primary imatinib resistance in chronic myelogenous leukemia are distinct from those in secondary imatinib resistance. *J Clin Oncol*. 2009;27(22):3642.
8. Bhamidipati PK, Kantarjian H, Cortes J, Cornelison AM, Jabbour E. Management of imatinib-resistant patients with chronic myeloid leukemia. *Ther Adv Hematol*. 2013;4(2):103-17.
9. Mohr A, Chatain N, Domoszlai T, et al. Dynamics and non-canonical aspects of JAK/STAT signalling. *Europ J Cell Biol*. 2012;91(6-7):524-32.
10. Mi T, Wang Z, Bunting K. The cooperative relationship between STAT5 and reactive oxygen species in leukemia: Mechanism and therapeutic potential. *Cancers*. 2018;10(10):359.
11. Ilaria RL, Van Etten RA. P210 and P190BCR/ABL induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. *J Biol Chemistry*. 1996;271(49):31704-10.
12. Gouilleux F, Wakao H, Mundt M, Groner B. Prolactin induces phosphorylation of Tyr694 of Stat5 (MGF), a prerequisite for DNA binding and induction of transcription. *The EMBO J*. 1994;13(18):4361-9.
13. Warsch W, Grundschober E, Sexl V. Adding a new facet to STAT5 in CML: multitasking for leukemic cells. *Cell cycle (Georgetown, Tex.)*. 2013;12(12):1813-4.
14. Constantinescu S.N., Girardot M., Pecquet C. Mining for JAK-STAT mutations in cancer. *Trends Biochem. Sci*. 2008;33:122-31.
15. Silva C.M. Role of STATs as downstream signal transducers in Src family kinase-mediated tumorigenesis. *Oncogene*. 2004;23:8017-23.
16. Ozawa Y, Williams AH, Estes ML, Matsushita N, Boschelli F, Jove R, List AF. Src family kinases promote AML cell survival through activation of signal transducers and activators of transcription (STAT) Leuk Res. 2008;32:893-903.
17. Bourgeais J, Gouilleux-Gruart V, Gouilleux F. Oxidative metabolism in cancer: A STAT affair? *JAKSTAT*. 2013;2:e25764.
18. Warsch W, Grundschober E, Berger A, Gille L, Cerny-Reiterer S, Tigan AS, Hoelbl-Kovacic A, Valent P, Moriggl R, Sexl V. STAT5 triggers BCR-ABL1 mutation by mediating ROS production in chronic myeloid leukaemia. *Oncotarget*. 2012;3:1669-87.
19. Casetti L, Martin-Lannere S, Najjar I, Plo I, Auge S, Roy L, Chomel JC, Lauret E, Turhan AG, Dusanter-Fourt I. Differential contributions of STAT5a and STAT5b to stress protection and tyrosine kinase inhibitor resistance of chronic myeloid leukemia stem/progenitor cells. *Cancer Res*. 2013;73:2052-8.
20. Schaller-Schönitz M, Barzan D, Williamson AJ, Griffiths JR, Dallmann I, Battmer K, Ganzer A, Whetton AD, Scherr M, Eder M. BCR-ABL Affects STAT5A and STAT5B Differentially. *PloS one*. 2014;9(5):e97243.
21. Chatain N, Ziegler P, Fahrenkamp D, et al. Src family kinases mediate cytoplasmic retention of activated STAT5 in BCR-ABL-positive cells. *Oncogene*. 2013;32(31):3587.
22. Furqan M, Akinleye A, Mukhi N, Mittal V, Chen Y, et al. (2013) STAT inhibitors for cancer therapy. *J Hematol Oncol*. 2006;90(8722):6-90.
23. Nelson EA, Walker SR, Weisberg E, et al. The STAT5 inhibitor pimozone decreases survival of chronic myelogenous leukemia cells resistant to kinase inhibitors. *Blood*. 2011;117(12):3421-9.